Abstract

Imagine you owned a patch of land, but couldn’t put up any fences. How would you remember what was yours and what belonged to your neighbor?

Many animal species protect areas of land, which we call their territories. By keeping competitors off their land, a territorial male is able to mate with any females that live there. As well as being strong enough to defend his territory, he has to be able to remember what belongs to him and what doesn’t! Otherwise he will risk getting into lots of fights with other territorial male neighbors.

A special part of the brain processes the memory of spaces and locations. We ran an experiment on lizards to see what caused this part of the brain to change size. The results were not what we expected!

Introduction

There are three different types of male side-blotched lizards (Uta stansburiana) (Fig. 1), and each has a different mating strategy. The biggest (orange-throated) males defend large territories, medium (blue-throated) males defend small territories, and the smallest (yellow-throated) males move around an even smaller area, and don’t defend any territory.

In an earlier study, we found that males with territories had bigger brains than those that didn’t defend any territory. The specific part of the brain that was bigger was the part associated with spatial memory (in lizards, this is the cortex). However, we didn’t know what was causing this relationship.

Many scientists believe that the hormone testosterone might be the link between the lizards’ use of space (their territorial strategy) and the brain.

We knew from previous studies that there were relationships between testosterone and the brain, and between testosterone and territoriality. What we wanted to understand better was whether testosterone could directly affect how the cortex grew, and if there was any difference depending on territorial strategy.

Based on what we expected, we tested two hypotheses in our experiment:

1. Increasing testosterone levels would increase the size of the cortex, the part of the brain associated with spatial memory, regardless of whether the lizards were territorial or not.

2. Testosterone would have a greater effect on territorial males than non-territorial males.
Methods

In our laboratory, we raised male lizards of each of the three different types. We put them in separate enclosures, and kept them warm and comfortable until they reached adulthood.

As other studies showed no difference in brain size between the orange and blue males, we created two groups based on whether the lizards had territories, or not:
- territorial lizards (orange- and blue-throated)
- non-territorial lizards (yellow-throated)

We took blood samples from all the lizards to measure starting testosterone levels. Then we split half the lizards from each group into a treatment and a control group:

Each treatment group received a testosterone implant that increased their testosterone levels. Each control group received a saltwater implant without any hormones. We used these lizards to compare the effects of the treatment group to a baseline. So ultimately, we had 4 different groups: 2 treatment groups and 2 control groups for both the territorial and the non-territorial lizards (Fig. 2).

We then waited two months to allow the hormone to work on the brain. After this time, we took blood samples, humanely killed the lizards, and removed their brains.

We cut the brains into extremely thin slices so that we could measure the size of the cortex (Fig. 3) and also count the number of new nerve cells in this region.

Finally, we analyzed the results to look for differences in testosterone levels, cortex sizes, and numbers of new nerve cells, based on territorial and treatment group.

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<thead>
<tr>
<th>Territorial lizards</th>
<th>Non-territorial lizards</th>
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<tr>
<td>Treatment group (extra Testosterone)</td>
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<tr>
<td>Control group (no added Testosterone)</td>
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Results

Testosterone levels were significantly higher in the lizards with implants of testosterone (treatment group) compared with those who just got salt water implants (control group).

We found no significant differences in the size of the cortex, or the number of new nerve cells, in either of the control groups (territorial and non-territorial), or in the non-territorial males that we gave extra testosterone to.

The territorial males that we gave extra testosterone to had significantly smaller cortices, with fewer new nerve cells (Fig. 4).
Discussion

Our results showed that testosterone can affect the spatial memory processing part of the brain, even when males are not living in differently sized areas. All of our lizards were in the same sized enclosures and were not carrying out their natural territorial strategies.

Territorial males (the orange and blue-throated lizards) seemed to be more sensitive to changes in testosterone levels than non-territorial (yellow-throated) males. However, the effect of testosterone on these lizards’ brains was negative when we expected it to be positive. We expected that giving the territorial lizards extra testosterone would make the part of the brain associated with spatial memory (the cortex) increase in size and have more new nerve cells in it, showing that it had recently grown. However, the opposite was the case: they had smaller cortices.

Other scientific studies showed that testosterone increased this part of the brain, so why were our findings different? We think that it might be due to differences in research methods, for example, how long you give the animals extra testosterone for, or which species you study. For this reason, we still can’t be certain what effect testosterone has on the brain, and must carry out more research to know for sure.

Which lizard males had the smallest sized cortex? Were they given extra testosterone or not, and were they territorial, or non-territorial?

Conclusion

Sometimes when you do a scientific experiment, you don’t get the results you expected. This is great science! You learn something new that you did not expect.

To make sure that we didn’t let our predictions affect our results, we measured our samples blind, without knowing which lizard’s brain we were looking at. It’s important to build these protective measures into scientific studies, so that the results we get are the real ones, and not just the ones we somehow influenced because we expected them.

If not, bias can have small effects on how we behave. If it’s unclear if there are 2 or 5 cells in a sample, what do you write down? If you knew the sample ‘should’ have more cells, might that make you more likely to write 5, rather than 2?
Glossary of Key Terms

**Bias** – an opinion that we have about something before we have looked at the evidence for it. In our study, we might have had a bias that testosterone would make the lizards’ brains bigger, which is why we took measures (and did a so-called blind study) to stop this opinion influencing our results.

**Blind study** – a study where we keep the identities of samples hidden from researchers until after we know the results, to prevent bias.

**Control group** – one of the tested groups in a scientific experiment, which is NOT exposed to any experimental treatment but is tested under the same conditions as the other groups. This helps the scientists confirm that the experimental treatment actually makes a difference, and is not just the result of natural variation, or random. In our study, the control group lizards were given an implant of salt water rather than testosterone.

**Cortex (plural = cortices)** – a small part of the reptile brain that controls how the animal processes spatial memory. In the mammalian brain, the hippocampus does the same job.

**Hormone** – a signaling substance that is created in a body and moved around in the blood and stimulates certain areas, organs or cells into action.

**Hypothesis** – a proposed explanation for an observed phenomenon or a prediction scientists make which can be tested and disproved. Hypotheses are usually based on observation or previous scientific knowledge.

**Significant** – a result that is likely not due to chance, but rather due to a real process. Scientists define a result as “significant” if it would happen by chance less than 5% of the time.

**Spatial memory** – the ability to remember information about spaces and locations. For example, your spatial memory helps you remember the layout of your hometown or the inside of your friend's house.

**Territory** – an area of land defended by an animal, or group of animals, from others of the same sex or species. The orange and blue-throated lizards in our study are territorial, meaning that they defend a territory.

**Territorial strategy** – describes whether animals have territories, and what they do with it. Do they defend a big one, a small one, or none at all?

**Testosterone** – a hormone that stimulates the development of some male sexual characteristics.

**Treatment group** – one of the tested groups in a scientific experiment, which IS exposed to the experimental treatment. In our study, we gave the “treatment group” lizards an implant of testosterone.

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**REFERENCES**

https://doi.org/10.3389/fnins.2017.00097

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The male side-blotched lizards have three different territorial strategies, which are also different mating strategies. How do you think each strategy works?

Hints: Female side-blotched lizards will mate with any of the three males. The yellow-throated males are considered “sneaker” males and also mimic female behaviors.

The side-blotched lizard is a great animal to study the effects of testosterone on territory size because there are different territorial strategies in the same sex of the species. Why does this make the study more accurate than studies across different species or sexes?

We only took blood samples once before we started the study and once before their death. If we had taken blood samples throughout the two-month period of the study, we could have had a more accurate knowledge of the hormone levels. Why do you think we didn’t do this?

The testosterone levels that we saw in the lizards in our study (before we gave them more) were lower than natural testosterone levels found in males in the wild. Why do you think this could have been?